Urinary erythropoietin concentrations after early short-term infusion of high-dose recombinant epo for neuroprotection in preterm neonates

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Abstract: BACKGROUND: High-dose recombinant human erythropoietin (rEpo) has first been administered in clinical trials for neuroprotection in very preterm neonates at high risk of brain injury and in (near-) term neonates with hypoxic-ischemic encephalopathy. However, recent trials in adults raised concerns about the safety of high-dose rEpo for neuro- and cardioprotection. OBJECTIVES: To evaluate the putative accumulation or renal leakage of Epo as a function of developmental stage after repetitive early short-term infusion of high-dose rEpo (3 × 3,000 U/kg within 42 h after birth; NCT00413946) for neuroprotection in very preterm infants. METHODS: Epo concentrations were measured using the ELISA technique in the first two consecutive urine specimens after each rEpo infusion. RESULTS: Renal Epo excretion was significantly higher in preterm infants with gestational ages <29 weeks than in more mature infants and reached up to 23% of the administered rEpo within 8 h after each infusion. The urinary Epo concentration did not increase after three repetitive infusions of high-dose rEpo. The ratio of urinary Epo to total protein concentrations was the same in infants with gestational ages <29 weeks and in those with gestational ages 29 weeks. CONCLUSIONS: Our data suggest that the higher renal Epo excretion in more immature infants may be attributed to a higher glomerular filtration leakage due to the lower maturation of the kidneys and argue against saturation kinetics after multiple doses of 3,000 U/kg rEpo. This information should be considered in future trials on the use of rEpo for neuroprotection in neonates.

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Urinary Erythropoietin Concentrations after Early Short-Term Infusion of High-Dose Recombinant Epo for Neuroprotection in Preterm Neonates

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Key Words
Erythropoietin · Neuroprotection · Brain injury · Preterm infant

Abstract
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Introduction

Animal models of neonatal brain injury have indicated significant neuroprotective effects of high-dose recombinant human erythropoietin (rEpo) treatment, leading to the first clinical trials in (near-) term neonates with hypoxic-ischemic encephalopathy (HIE) and in very preterm neonates at high risk of intraventricular hemor-

C.D. and J.L. contributed equally to this study.
This trial has been registered at www.clinicaltrials.gov (identifier NCT00413946).

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Table 1. Demographic data

Demographic data	rEpo (n = 42)	Placebo (n = 33)	Difference[1]	Odds ratio[1]	p value
Girls, n	16 (38%)	9 (27%)		1.641 (0.611–4.404)	0.32
Mean gestational age ± SD, weeks + days	29 1/7 ± 1 6/7	29 2/7 ± 1 5/7	0 2/7 (–0 4/7 to 1 4/7)		0.56
Mean birth weight ± SD, g	1,211 ± 327	1,241 ± 341	30 (–184 to 124)		0.70
Mean z-score ± SD	0.06 ± 0.78	0.005 ± 0.93	0.06 (–0.33 to 0.45)		0.76
Mean birth head circumference ± SD, cm	26.6 ± 2.0	27.4 ± 2.3	–0.7 (–1.7 to 2.1)		0.12
Mean z-score ± SD	–0.10 ± 0.61	0.09 ± 0.85	–0.20 (–0.54 to 0.13)		0.23
Chorioamnionitis, n	15 (36%)	8 (24%)		1.736 (0.628–4.795)	0.28
Antenatal steroids, n	32 (76%)	25 (75%)		1.024 (0.352–2.975)	0.96
Cesarean section, n	36 (85%)	33 (100%)		0.083 (0.004–1.545)	0.09
Mean pH umbilical artery ± SD	7.31 (0.09%)	7.35 (0.07%)	–0.02 (–0.06 to 0.01)		0.26
Mean maximum negative base excess ± SD	–5.1 (2.9%)	–4.0 (2.9%)	–1.1 (–2.4 to 0.3)		0.12
Apgar score at 5 min ≤5, n	8 (19%)	6 (18%)		1.058 (0.327–3.42)	0.97
Median CRIB score[2]	2 (4–1)	1 (4–1)	1		
Catecholamine treatment, n	4 (10%)	2 (6%)		1.631 (0.28–9.506)	0.58
Mechanical ventilation, n	13 (31%)	11 (33%)		0.896 (0.338–2.378)	0.82

[1] Figures shown in parentheses are 95% confidence intervals. [2] Figures shown in parentheses are interquartile ranges.

rhage or periventricular leukomalacia [1–4]. Important questions on the dosing strategy (optimal timing, dosage, route and repetitive infusion) remain open, and long-term follow-up analysis on secondary outcome measures needs to be considered. Although both trials using early high-dose rEpo in very preterm infants did not show any significant differences in safety parameters or any toxic side effects as compared with the placebo control groups [1, 2], concerns regarding adverse effects associated with high-dose rEpo in adults with stroke [5], cardiovascular disorders [6], renal failure [7], or cancer [8] prompted the FDA to issue a temporary hold on clinical trials on tissue-protective effects of high-dose rEpo [9].

Among the studies investigating early high-dose rEpo for neuroprotection in preterm infants, the US phase II trial on 'High-Dose Erythropoietin in Extremely Premature Infants to Prevent/Attenuate Brain Injury' (NCT00589953) was registered in 2007, but placed on FDA hold until 2010, and has still not been initiated yet. A novel US phase II/III 'Trial of Erythropoietin Neuroprotection in Extremely Preterm Infants (PENUT; NCT013778273) is supposed to start recruitment in July 2012. The previous US 'Phase I/II trial of high-dose rEpo in extremely low birth weight infants' (IND12656) provided data on Epo plasma levels after early high-dose intravenous rEpo. Considering the ongoing discussion about the safety of rEpo in neonates [10–13], we evaluated the putative accumulation or renal leakage of Epo as a function of developmental stage following repetitive early short-term infusion of high-dose rEpo for neuroprotection in very preterm infants included in the ongoing Swiss phase II trial (NCT00413946).

Methods

269 urine specimens (rEpo n = 154; placebo n = 115) were obtained from 75 neonates (rEpo n = 42; placebo n = 33) included in this randomized, controlled double-blind, multicenter trial on the use of high-dose rEpo (epoetin-beta, Hoffmann-La Roche) for neuroprotection in very preterm infants (NCT00413946). Urine specimens were collected from all consecutive patients recruited at the Zurich study center from January 2006 to October 2009. In 3 patients, we were unable to collect at least two urine specimens, which was the only putative reason for excluding patients from the subanalysis. These patients did not suffer from HIE. Their demographic data are summarized in table 1. Frequencies of traits were compared by odds ratios with 95% confidence intervals. Continuous measurements that followed a normal distribution are summarized as means and standard deviation plus 95% confidence intervals for the difference between means. The CRIB score is reported as medians and interquartile ranges [1]. Patients were treated either with 3,000 U epoetin-beta per kilogram body weight, administered as a short-term infusion over a period of 10 min at 1–3, 12–18 and 36–42 h after birth or with an equivalent volume of 0.9% NaCl. The first two consecutive urine specimens after each rEpo infusion were collected using polyethylene tubes in male and sterile gloves in female infants. Corresponding plasma/serum or cerebrospinal fluid specimens were not taken to avoid unnecessary blood loss or lumbar taps. Epo concentrations

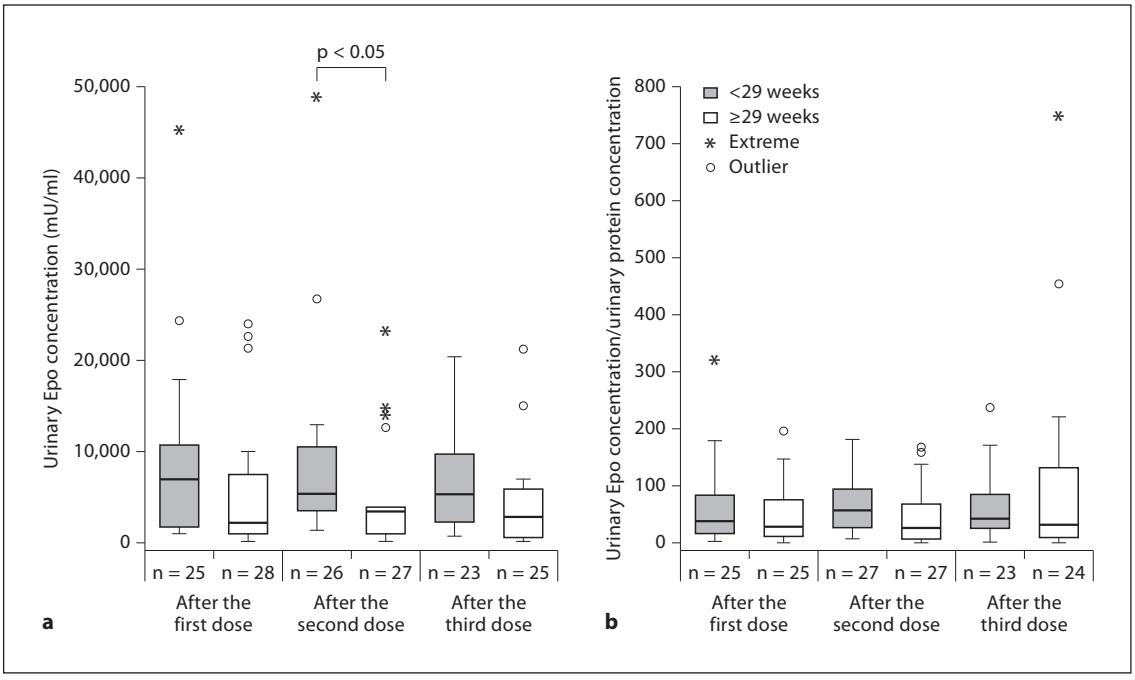

Fig. 1. Urinary Epo concentrations (**a**) and ratio of urinary Epo to urinary total protein concentrations (**b**) in very preterm infants, who received 3,000 U epoetin-beta per kilogram body weight as short-term infusion. Patients were allocated to two groups according to gestational ages <29 or ≥29 weeks. Numbers represent the number of urine specimens investigated. Statistical analysis was performed using ANOVA.

were determined in duplicate using the Quantikine Human Epo Immunoassay (R&D Systems), with a lower detection limit of 2.5 mU/ml; the intra-assay variability was <2%. Total urinary protein concentrations were determined using the Microassay Protocol of the Quick Start™ Bradford Protein Assay (Bio-Rad Laboratories) according to the manufacturer's instructions. The study was blinded in that only the study pharmacist had access to the patients' allocation to the rEpo or placebo group. After measuring the urinary Epo concentrations, the anonymous data were sorted into the rEpo or placebo group by the pharmacist. All investigators remained strictly blinded to this allocation in order not to prejudice any aspect of long-term follow-up examination. As to the renal development and functional adaptation of the kidneys [14–16], patients were allocated to two groups according to their gestational age at birth: group 1: 26 0/7–28 6/7 weeks (rEpo n = 21; placebo n = 14); group 2: 29 0/7–31 6/7 (rEpo n = 21; placebo n = 19). For statistical analysis, the Mann-Whitney U test (two-sided) or the ANOVA were applied as indicated. A p value <0.05 was considered to be significant.

Results

Epo was undetectable in 86.2% of the urine samples of the placebo group. In the remaining Epo-positive samples, the Epo concentration ranged between 2.5 and 18.1 mU/ml. In the rEpo group, 98.7% of urine samples tested positive for Epo. The urinary Epo concentrations (median 3.930; range <2.5–48.940 mU/ml) in the rEpo group were significantly higher ($p < 0.001$) than in the placebo group. Infants with gestational ages <29 weeks had significantly higher urinary Epo concentrations than more mature infants ($p = 0.03$). In both subgroups, the urinary Epo concentrations did not significantly differ in the first and the second urine sample taken after each of the three repetitive rEpo infusions. Nor was there a significant change in urinary Epo concentrations after the first, second or third infusion of high-dose rEpo (fig. 1a). Notably, outlying or extreme values were not found to be dependent on any of the analyzed parameters, including the finding of oligohydramnios prior to birth, presence of chorioamnionitis, or treatment with antibiotics or catecholamines. None of the patients were treated with indomethacin prior to the third infusion of rEpo. The ratios between urinary Epo and total urinary protein concentrations were almost equal in both subgroups; this was also the case after repetitive rEpo infusions (fig. 1b). Longitudinal analysis of individual patients indicated no changes in the peak and trough concentrations after the

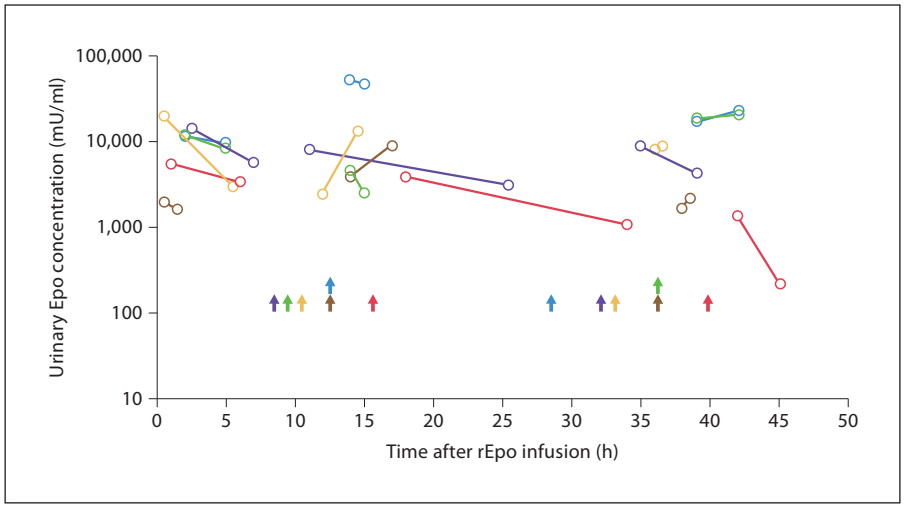

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Fig. 2. Longitudinal analysis of urinary Epo concentrations in 6 infants with gestational ages <29 weeks at birth, treated with short-term infusions of 3,000 U epoetin-beta per kilogram body weight. Circles indicate urinary Epo concentrations in two consecutive specimens taken after the first, second and third infusion of rEpo. The arrows, colored to follow up each individual patient, indicate the time point of the second and third rEpo infusion. The first dose was given within 3 h after birth. For colors, see online version.

repetitive rEpo infusions (fig. 2). The patients shown in figure 2 have been selected for the graphic presentation since collection of two consecutive urine specimens after each rEpo infusion could be successfully completed only in these 6 patients.

In infants with gestational ages <29 weeks treated for neuroprotection with a short-term infusion of 3,000 U rEpo per kilogram body weight, up to 23% (median 2.8%) of rEpo was secreted in the urine within the first 8 h. This urinary Epo excretion reached only up to 9.4% (median 1.2%) in infants with gestational ages ≥29 weeks.

Discussion

Our study provides the first data on urinary Epo concentrations in preterm infants treated with high-dose rEpo for neuroprotection. Compared with the placebo group, significantly higher urinary Epo concentrations were detected in the rEpo group. In the rEpo group, urinary Epo concentrations were significantly higher in preterm infants born at gestational ages <29 weeks compared with more mature infants ($p < 0.05$; fig. 1a). This finding parallels of higher mean plasma creatinine concentrations within the first 48 h and with a lower plasma creatinine clearance (as a measure of the glomerular filtration rate) in very immature (<29 weeks of gestation) versus more mature infants [15, 16]. Due to these fundamental developmental changes and variable maternal/perinatal factors, the amount of early Epo excretion cannot be directly correlated with renal function based on plasma creatinine levels, or the glomerular filtration rate within the first 48 h after birth. However, equal ratios between urinary Epo and total urinary protein concentrations (fig. 1b) indicate that higher Epo excretion in the more immature infants may result from a greater protein leakage in glomerular filtration.

Interestingly, we were also able to measure low, but detectable Epo concentrations (max. 18.1 mU/ml) in a few urine samples of the placebo group (13.8%). This observation may be explained by the fact that fetal distress (anemia, hypoxia, preeclampsia, maternal type 1 diabetes) induces sufficient endogenous Epo production, which leads to detectable or even high Epo concentrations in the amniotic fluid [17]. Importantly, baseline urinary Epo concentrations in healthy adults also range between 9.3 and 23 mU/ml [18].

Nonlinear pharmacokinetics of early high-dose rEpo for neuroprotection in very premature infants was determined based on repetitive analysis of plasma Epo concentrations in very preterm infants in the US phase I/II clinical trial [2], indicating a decreasing clearance from the lowest dose (17.3 ml/kg/h; rEpo 500 U/kg) to the highest dose (8.2 ml/kg/h; rEpo 2,500 U/kg). For doses up to 2,500 U/kg/day, administered on 3 consecutive days, peak and trough concentrations did not significantly change after the second dose [2]. Our data parallel these results by showing similar high urinary Epo concentrations after the first, second and third high-dose rEpo infusions, and thus support the conclusion that there is no evidence for rEpo accumulation after multiple doses of 3,000 U/kg rEpo administered to very preterm infants; using a drug with nonlinear pharmacokinetics would otherwise be an important issue.

Of note, the excreted Epo in relation to the infused rEpo dose decreased from up to 23% within the first 8 h after the first short-term infusion of rEpo in infants with gestational ages <29 weeks to a maximum of 9.4% in infants with gestational ages ≥29 weeks. A similar amount of Epo excreted in the urine (3–10%) has been detected in healthy adults who received low-dose rEpo (200 U/kg/day s.c.) [18]. In preterm infants treated with a low dose of 250 U/kg rEpo for anemia of prematurity, up to 2.8% of Epo was excreted in the urine within the first 8 h after the first intravenous administration [19]. These data argue against saturation kinetics. Rather, they suggest the conclusion that a higher Epo excretion in the more immature infants is caused by the lower maturation of their kidneys.

Regarding the efficacy of early high-dose Epo, the lower renal Epo excretion in infants with gestational ages >29 weeks may be important.

Both published trials [3, 4] and the ongoing, but not active trials (NCT00719407; NCT00513240, NCT00491413) on the effects of rEpo in (near-) term infants suffering from HIE follow different conceptual strategies regarding the timing, dosage, route and repetition of rEpo administration. Since these infants frequently suffer from oliguria or anuria during the first days after birth, we recommend the assay of urinary (and if possible plasma) Epo concentrations and creatinine clearance in the ongoing trials in order to gain insight into the pharmacokinetics of early high-dose rEpo treatment for neuroprotection in HIE. Initial accumulation or later increased leakage may contribute to the finding that rEpo improved the long-term outcome only in infants with moderate, but not with severe, HIE [3].

In an approach that combines therapeutic hypothermia with rEpo or darbepoietin (a highly glycosylated Epo derivate) treatment for neuroprotection after HIE (NCT01471015), it should be considered that hypothermia may further reduce the glomerular filtration rate [20, 21] and also modify renal rEpo protein leakage or accumulation. Notably, in adults with out-of-hospital cardiac arrest, early high-dose rEpo plus hypothermia resulted in a higher incidence of thrombocytosis as a putative side effect of rEpo [22].

In conclusion, the difference in glomerular filtration leakage of rEpo should be considered as a variable in the saturation kinetics of high-dose rEpo in very immature premature infants and thus as a putative factor influencing the neuroprotective benefit of rEpo.

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Disclosure Statement

All authors declare that they have no potential, perceived, or real conflict of interest to disclose and no financial relationships relevant to this study.

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